SUPPLEMENTAL AMENDMENT
U.S. APPLN. NO. 09/856,374

ATTY DKT Q64360

REMARKS

I. Endothelial Cells

In the Amendment filed September 15, 2004 in this application, Applicants argued that the present invention is not obvious, because although Isner et al. and Morishita et al. teach treating ischemia in cardiac and skeletal muscle with VEGF and HGF genes via HVJ-liposomes, muscle tissue is not representative of brain tissue for purposes of gene therapy.

During the October 13, 2004 Examiner Interview, the Examiner contended that even though muscle and brain are different tissues, VEGF and HGF affect endothelial cells, which are the same in both types of tissue. Therefore the Examiner asserted that successful use of VEGF and HGF via HVJ-liposomes for gene therapy in vascular endothelial cells in muscle teaches or suggests successful use for gene therapy in vascular endothelial cells in brain.

In response, Applicants submit that endothelial cells in the brain are fundamentally different from endothelial cells in other organs. Therefore, a person of ordinary skill in the art would not have reasonably expected VEGF and HGF to be effectively expressed in endothelial cells in the brain.

In Garlanda and Dejana, *Heterogeneity of Endothelial Cells*, Arteriosclerosis, Thrombosis and Vascular Biology, Vol. 17, No. 7, July 1997, 1193-1202, the authors discuss the heterogeneity of endothelial cells in detail. For example, the authors explain that

> One of the important determinants of endothelial cell differentiation is the local environment, and especially the interaction with surrounding cells. This interaction may occur through the release of soluble cytokines, cell-to-cell adhesion and communication, and the synthesis of matrix proteins on which the endothelium adheres and grows. The acquisition and maintenance of specialized properties by endothelial cells is important in the functional homeostasis of the different organs. For instance, in the brain, alteration of the blood-brain barrier properties may have important consequences on brain functional integrity. Abstract, page 1193.

Furthermore, the authors state that a "remarkable heterogeneity" in different organs is a "unique characteristic" of endothelial cells in general. *Id.* For example, identifying markers expressed by endothelial cells can be specific for endothelial cells of different origins, and "it is not unusual that the same protein may be expressed, with different degrees and types of glycosylation, in different vascular regions." Page 1194.

With regard to brain endothelial cells in particular, Garlanda and Dejana note at page 1195 that endothelium of the brain are among the several examples of specialized endothelial cells. For example, the authors explain that during differentiation, brain endothelial cells "not only acquire specific markers, but also lose, or express to a low degree, molecules that are otherwise present in other types of endothelium. Page 1197. The authors also point out that due to the unique location of the brain microvasculature at the interface between blood and the central nervous system, these cells have "specific protective properties that strictly regulate the infiltration of plasma components and circulating cells into the brain." Page 1197. The authors

point to well-developed tight junctions, well-established cell polarization, very selective intracellular transport systems, and a very low pinocytotic activity as examples of the unique characteristics of brain endothelial cells. Id.

Risau, *Differentiation of Endothelium*, The FASEB Journal, Vol. 9, July 1995, 926-933, also supports the unique character of brain endothelial cells, stating for example that "the unique features of brain endothelial cells allow precise control over the substances that leave or enter the brain." Page 931.

In conclusion, a person of ordinary skill in the art would recognize that endothelial cells in different organs are remarkably heterogeneous. In particular, endothelial cells in the brain are quite fundamentally different from those in other organs. A person of ordinary skill in the art would also expect that some if not all of the unique characteristics of brain endothelial cells would affect gene delivery, gene expression, and ultimately the effectiveness of gene therapy in the brain. Thus, the teachings of the references cited by the Examiner clearly do not provide a reasonable expectation that the present invention would be successful.

II. HVJ-Liposomes

Applicants have also argued that because none of the cited references teaches gene therapy via HVJ-liposome-mediated delivery to the brain, a person of ordinary skill in the art would not have reasonably expected this delivery system to be operable in the brain. The Examiner contended that because this delivery system has been used for successful gene therapy to endothelial cells in muscle (citing Isner and Morishita) and to endothelial cells in lung (citing

Yonemitsu), there is no reason to expect that HVJ-liposomes would not be effective for gene therapy to endothelial cells in the brain.

In the interview, Applicants' representatives pointed out that Yonemitsu teaches away from HVJ-liposomes, and describes the use of a different gene transfer system, "HVJ-cationic liposomes," for *in vivo* gene transfer to lung airway epithelium. Applicants representatives also noted that Yonemitsu teaches that HVJ-liposomes were ineffective for this purpose, and that the reference points to other problems with the HVJ-liposome vector system (these arguments are also set forth at page 9 of the Amendment filed September 15, 2004).

In response to Applicants' arguments, the Examiner agreed that if Applicants could distinguish the HVJ-cationic liposomes used by Yonemitsu from the HVJ-liposomes of the present invention, this would provide persuasive evidence that a person of ordinary skill in the art would not have reasonably expected HVJ-liposomes to function in the brain.

Accordingly, Applicants provide the following evidence that the HVJ-liposomes of the present invention are different from the HVJ-cationic liposomes reported in Yonemitsu, and that the HVJ-liposomes of the present invention are equivalent to the HVJ-liposomes found to be ineffective in Yonemitsu.

Applicants note that the "conventional" HVJ-liposomes found to be ineffective in Yonemitsu are described in Kaneda et al., *The Improved Efficient Method for Introducing Macromolecules into Cells Using HVJ (Sendai Virus) Liposomes with Gangliosides*, Exp. Cell Research 173:56-69 (1987) and other references cited by Yonemitsu (see page 631, and cited references 7-9).

Kaneda describes preparation of HVJ-liposomes from the mixture of PS (phosphatidylserine), PC (phosphatidylcholine) and Chol (cholesterol) in a weight ratio of 1:4.8.2. The preparation disclosed in Kaneda is identical to the HVJ-liposome preparation method described in the present specification at page 26, line 25 to page 27, line 7.

In contrast to the HVJ-liposomes of the present invention (found to be ineffective in Yonemitsu), the "HVJ-cationic liposomes" successfully used in Yonemitsu are modified with the positively charged lipid N-(α-trimethylammonioacetyl)-didodecyl-D-glutamate chloride (TMAG) (see pages 632, 636, and 637).

Accordingly, a person of ordinary skill in the art would not have reasonably expected the "conventional" HVJ-liposomes of the present invention to be successful in gene therapy in the brain.

In view of the arguments and evidence presented above, Applicants respectfully request reconsideration and withdrawal of the obviousness rejection.

III. Conclusion

In view of the above, reconsideration and allowance of this application are now believed to be in order, and such actions are hereby solicited. If any points remain in issue which the Examiner feels may be best resolved through a personal or telephone interview, the Examiner is kindly requested to contact the undersigned at the telephone number listed below.

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Respectfully submitted,

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